

Amendments to the Claims

The listing of claims will replace all prior versions, and listings of claims in the application.

1. (Currently Amended) A multiple inducible gene regulation system consisting of two orthogonal gene regulation systems, wherein:

a) each of said orthogonal gene regulation systems comprises:

i) a polynucleotide encoding a receptor complex comprising:

A) a DNA binding domain;

B) an ecdysone receptor ligand binding domain; and

C) a nuclear receptor ligand binding domain capable of forming a dimer with the ecdysone receptor ligand binding domain; and

D) Θ a transactivation domain; and

ii) a polynucleotide comprising a DNA sequence encoding a polypeptide operatively linked to a response element,

wherein binding of the DNA binding domain to the response element results in expression of the polypeptide.

2. (Previously Presented) The multiple inducible gene regulation system of claim 1, wherein each operable gene regulation system comprises

a) i) a first gene expression cassette comprising a polynucleotide that encodes a

polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and an ecdysone receptor ligand binding domain, and

ii) a second gene expression cassette comprising:

A) a response element recognized by the DNA-binding domain;

B) a promoter that is activated by the transactivation domain; and

C) a gene whose expression is to be modulated;

b) i) a first gene expression cassette comprising a polynucleotide that encodes a polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and an ecdysone receptor ligand binding domain,

ii) a nuclear receptor ligand binding domain selected from the group consisting of a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, and a chimeric ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or ultraspiracle protein ligand binding domain, and

iii) a second gene expression cassette comprising:

A) a response element recognized by the DNA-binding domain;

B) a promoter that is activated by the transactivation domain; and

C) a gene whose expression is to be modulated; or

c) i) a first gene expression cassette comprising a polynucleotide that encodes a first polypeptide comprising a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated and an ecdysone receptor ligand binding domain,

ii) a second gene expression cassette comprising a polynucleotide that encodes a second polypeptide comprising a transactivation domain and an ecdysone receptor ligand binding domain, and

iii) a third gene expression cassette comprising:

A) a response element recognized by the DNA-binding domain;

B) a promoter that is activated by the transactivation domain; and

C) a gene whose expression is to be modulated.

3. (Original) A virus comprising the multiple gene regulation system of claim 1.

4. (Currently Amended) An isolated host cell comprising the multiple gene regulation system of claim 1.

5. (Canceled)

6. (Canceled)

7. (Previously Presented) The multiple inducible gene regulation system of claim 1, wherein said receptor complex is a non-mammalian receptor complex.

8. (Canceled)

9. (Currently Amended) A multiple inducible gene regulation system consisting of two orthogonal gene regulation systems wherein:

a) each of said gene regulation systems comprises:

i) a receptor complex comprising:

A) a DNA binding domain;

B) an ecdysone receptor ligand binding domain; and

C) a nuclear receptor ligand binding domain capable of forming a dimer with the ecdysone receptor ligand binding domain; and

D E) a transactivation domain; and

ii) a polynucleotide comprising:

an exogenous or endogenous gene and a response element;

wherein:

A) the exogenous or endogenous gene is under the control of the response element;

and

B) binding of the DNA binding domain to the response element in the presence of a ligand results in activation of the gene.

10. (Previously Presented) The multiple inducible gene regulation system of claim 9, wherein each operable gene regulation system comprises:

- a) i) a polypeptide comprising a transactivations domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and an ecdysone receptor ligand binding domain, and
 - ii) a gene expression cassette comprising:
 - A) a response element recognized by the DNA-binding domain of the polypeptide of a)i);
 - B) a promoter that is activated by the transactivation domain of the polypeptide of a)i); and
 - C) a gene whose expression is to be modulated;
- b) i) a polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and an ecdysone receptor ligand binding domain,
 - ii) a nuclear receptor ligand binding domain selected from the group consisting of a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, and a chimeric ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or ultraspiracle protein ligand binding domain, and

- iii) a gene expression cassette comprising:
 - A) a response element recognized by the DNA-binding domain of the polypeptide of b)i);
 - B) a promoter that is activated by the transactivation domain of the polypeptide of b)i); and
 - C) a gene whose expression is to be modulated; or
- c) i) a first polypeptide comprising a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated and an ecdysone receptor ligand binding domain,
 - ii) a second polypeptide comprising a transactivation domain and a nuclear steroid receptor ligand binding domain, and
 - iii) a gene expression cassette comprising:
 - A) a response element recognized by the DNA-binding domain of the first polypeptide of c)i);
 - B) a promoter that is activated by the transactivation domain of the second polypeptide of c)ii); and
 - C) a gene whose expression is to be modulated.

11. (Canceled)

12. (Currently Amended) An isolated host cell comprising the multiple gene regulation system of claim 9.

13. - 49. (Canceled)

50. (Previously Presented) A vector comprising the multiple inducible gene regulation system of claim 1.

51. (Previously Presented) The isolated host cell of claim 4, wherein the host cell is a bacterial cell, a fungal cell, a yeast cell, a plant cell, an animal cell, a mammalian cell, a mouse cell, or a human cell.

52. (Previously Presented) The isolated host cell of claim 51, wherein the host cell is a human cell.

53. (Previously Presented) The isolated host cell of claim 12, wherein the host cell is a bacterial cell, a fungal cell, a yeast cell, a plant cell, an animal cell, a mammalian cell, a mouse cell, or a human cell.

54. (Previously Presented) The isolated host cell of claim 53, wherein the host cell is a human cell.

55. (New) The multiple inducible gene modulation system of claim 1, wherein said nuclear receptor ligand binding domain is selected from the group consisting of a vertebrate retinoid X receptor ligand binding domain; an invertebrate retinoid X receptor ligand binding domain; an ultraspiracle protein ligand binding domain; and a chimeric

ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain.

56. (New) The multiple inducible gene modulation system of claim 1, wherein said ecdysone receptor ligand binding domain is selected from the group consisting of a Lepidopteran ecdysone receptor ligand binding domain, a Dipteran ecdysone receptor ligand binding domain, an Arthropod ecdysone receptor ligand binding domain, an ecdysone receptor ligand binding domain, a Homopteran ecdysone receptor ligand binding domain, a Hemipteran ecdysone receptor ligand binding domain, a spruce budworm *Choristoneura fumiferana* ecdysone receptor ligand binding domain, a yellow meal worm *Tenebrio molitor* ecdysone receptor ligand binding domain, a tobacco hornworm *Manduca sexta* ecdysone receptor ligand binding domain, a tobacco budworm *Heliothis virescens* ecdysone receptor ligand binding domain, a golmidge *Chironomus tentans* ecdysone receptor ligand binding domain, a silkworm *Bombyx mori* ecdysone receptor ligand binding domain, a squinting bush brown *Bicyclus anynana* ecdysone receptor ligand binding domain, a buckeye *Junonia coenia* ecdysone receptor ligand binding domain, a fruit fly *Drosophila melanogaster* ecdysone receptor ligand binding domain, a yellow fever mosquito *Aedes aegypti* ecdysone receptor ligand binding domain, a blowfly *Lucilia capitata* ecdysone receptor ligand binding domain, a sheep

blowfly *Lucilia cuprina* ecdysone receptor ligand binding domain, a blowfly *Calliphora vicinia* ecdysone receptor ligand binding domain, a Mediterranean fruit fly *Ceratitis capitata* ecdysone receptor ligand binding domain, a locust *Locusta migratoria* ecdysone receptor ligand binding domain, an aphid *Myzus persicae* ecdysone receptor ligand binding domain, a fiddler crab *Celuca pugilator* ecdysone receptor ligand binding domain, an ixodid tick *Amblyomma americanum* ecdysone receptor ligand binding domain, a white fly *Baumechia argentifoli* ecdysone receptor ligand binding domain.

57. (New) The multiple inducible gene modulation system of claim 22, wherein said ecdysone receptor ligand binding domain is a spruce budworm *Choristoneura fumiferana* ecdysone receptor ligand binding domain.

58. (New) The multiple inducible gene modulation system of claim 1, wherein said DNA binding domain is selected from the group consisting of a GAL4 DNA binding domain, a LexA DNA binding domain, a transcription factor DNA binding domain, a Group H nuclear receptor member DNA binding domain, a steroid/thyroid hormone nuclear receptor superfamily member DNA binding domain, a bacterial LacZ DNA binding domain, DNA binding domain, an ecdysone receptor DNA binding domain.

59. (New) The multiple inducible gene modulation system of claim 1, wherein said transactivation domain is selected from the group consisting of a Group H nuclear receptor member transactivation domain, a steroid/thyroid hormone nuclear receptor transactivation domain, a polyglutamine transactivation domain, a basic or acidic amino

acid transactivation domain, a VP16 transactivation domain, a GAL4 transactivation domain, an NF-KB transactivation domain, a BP64 transactivation domain, a B42 acidic transactivation domain, a p65 transactivation domain.

60. (New) The virus of claim 3, wherein said virus is an adenovirus.

61. (New) The vector of claim 50, wherein said vector is a plasmid.

62. (New) The vector of claim 50, wherein said vector is an expression vector.

63. (New) The vector of claim 50, wherein said vector is a viral vector.

64. (New) The vector of claim 63, wherein said vector is an adenovirus vector.

65. (New) The isolated host cell of claim 4, wherein said cell is a mammalian cell.

66. (New) The isolated host cell of claim 65, wherein said mammalian cell is selected from the group consisting of a hamster cell, a murine cell, a monkey cell, a mouse cell, a rat cell, a rabbit cell, a cat cell, a dog cell, a bovine cell, a goat cell, a pig cell, a horse cell, a sheep cell, a monkey cell and a chimpanzee cell.

67. (New) The isolated host cell of claim 4, wherein the host cell is selected from the group consisting of an *Aspergillus* cell, a *Trichoderma* cell, a *Saccharomyces* cell, a *Pichia* cell, a *Candida* cell, and a *Hansenula* cell.

68. (New) The isolated host cell of claim 4, wherein the host cell is selected from the group consisting of a *Synechocystis* cell, a *Synechococcus* cell, a *Salmonella* cell, a *Bacillus* cell, an *Acinetobacter* cell, a *Rhodococcus* cell, a *Streptomyces* cell, an *Escherichia* cell, a *Pseudomonas* cell, a *Methylomonas* cell, a *Methylobacter* cell, an *Alcaligenes* cell, a *Synechocystis* cell, an *Anabaena* cell, a *Thiobacillus* cell, a *Methanobacterium* cell and a *Klebsiella* cell.

69. (New) The isolated host cell of claim 4, wherein the host cell is a plant cell.

70. (New) The isolated host cell of claim 69, wherein the plant cell is selected from the group consisting of an apple cell, an *Arabidopsis* cell, a bajra cell, a banana cell, a barley cell, a bean cell, a beet cell, a blackgram cell, a chickpea cell, a chili cell, a cucumber cell, an eggplant cell, a favabean cell, a maize cell, a melon cell, a millet cell, a mungbean cell, an oat cell, an okra cell, a *Panicum* cell, a papaya cell, a peanut cell, a pea cell, a pepper cell, a pigeonpea cell, a pineapple cell, a *Phaseolus* cell, a potato cell, a pumpkin cell, a rice cell, a sorghum cell, a soybean cell, a squash cell, a sugarcane cell, a sugarbeet cell, a sunflower cell, a sweet potato cell, a tea cell, a tomato cell, a tobacco cell, a watermelon cell, and a wheat cell.